Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/US05/003282

International filing date: 03 February 2005 (03.02.2005)

Document type: Certified copy of priority document

Document details: Country/Office: EP
Number: 04250558.6
Filing date: 03 February 2004 (03.02.2004)

Date of receipt at the International Bureau: 01 June 2005 (01.06.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

Europäisches Patentamt	European Patent Office	Office européen des brevets

US/05/3282

Bescheinigung | Certificate | Attestation

Die angehefteten Unterlagen stimmen mit der ursprünglich eingereichten Fassung der auf dem nächsten Blatt bezeichneten europäischen Patentanmeldung überein.

The attached documents are exact copies of the European patent application described on the following page, as originally filed.

Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécifiée à la page suivante.

Patentanmeldung Nr. | Patent application No. | Demande de brevet n°

04250558.6

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

R C van Dijk

Anmeldung Nr:
Application no.: 04250558.6
Demande no:

Anmeldetag:
Date of filing: 03.02.04
Date de dépôt:

Anmelder/Applicant(s)/Demandeur(s):

Cargill Incorporated
15407 McGinty Road West
Wayzata,
Minnesota 55391
ETATS-UNIS D'AMERIQUE

Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
Si aucun titre n'est indiqué se referer à la description.)

Protein concentrate and an aqueous stream containing water-soluble carbohydrates

In Anspruch genommene Priorität(en) / Priority(ies) claimed /Priorité(s) revendiquée(s)
Staat/Tag/Aktenzeichen/State/Date/File no./Pays/Date/Numéro de dépôt:

Internationale Patentklassifikation/International Patent Classification/
Classification internationale des brevets:

A23K/

Am Anmeldetag benannte Vertragstaaten/Contracting states designated at date of filing/Etats contractants désignées lors du dépôt:

AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LU MC NL
PT RO SE SI SK TR LI

PROTEIN CONCENTRATE AND AN AQUEOUS STREAM CONTAINING WATER-SOLUBLE CARBOHYDRATES

Disclosed herein are protein concentrates and aqueous streams containing water-soluble carbohydrates (co-products) and methods of preparing them.

For over 100 years corn wet milling has been used to separate corn kernels into products such as starch, protein, fiber and oil. Corn wet milling is a two stage process: (a) a steeping process to soften the corn kernel and to facilitate the next step; (b) a wet milling process resulting in purified starch and different co-products such as oil, fiber, and protein. In general, starch recoveries are between 90 to 96 %. The remainder of the starch is found in the different co-products.

US patent 2003/0070673 to Liaw et al., US patents 4,144,087 and 4,244,748 to Chwalek et al., EP patent 0 506 233 to Chie-Ying, US patent 3,928,631 to Freeman et al., US patent 4,960,705 to Johann et al., Patent WO 93/12667 to Cook et al., US patent 4,361,651 to Keim, WO patent 02/067698 to Kvist et al., US patents 5,773,076 and 5,968,585 to Liaw et al. relate to wet-milling processes that produce various products.

The disclosed process provides methods of making aqueous streams containing water-soluble carbohydrates and protein concentrates.

In some embodiments these methods involve contacting a stream that has previously been used in a wet-milling process (wet-mill stream) with protein-containing material that has also been obtained from a wet-milling process. These two components are then additionally contacted with carbohydrate hydrolyzing enzymes (carbohydrases) that break-down the starch and/or non-starch complex carbohydrates, such as fiber, into water soluble carbohydrates. The resulting protein concentrate is then separated from the aqueous stream, thus resulting in two products an aqueous stream that has an increased level of water-soluble carbohydrates (increased meaning greater than prior to contact with the protein-containing material and the hydrolyzing enzymes) and protein concentrate that has an increased protein concentration (increased meaning greater than prior to contacting the wet-mill stream and the carbohydrases).

In other embodiments, a protein concentrate and an aqueous stream containing water-soluble carbohydrates can be made from grain by contacting one or more protein containing materials with one or more wet-mill streams and one or more carbohydrases and then separating the resulting protein concentrate from the resulting aqueous stream containing water-soluble carbohydrates. The separation can be accomplished using any method known in the art for example membrane separation, centrifugation, floatation, and the like. In another embodiment, a membrane filtration is performed before or after the separation of the protein concentrate and the aqueous stream. The protein concentration of the protein concentrate can be further increased by defatting the protein containing material. Defatting can be accomplished by contacting the protein-containing material with a solvent and/or an enzyme.

In some embodiments the protein-containing material comprises gluten, and in yet other embodiments the protein-containing material can be bleached using enzymes and/or chemicals.

As mentioned below, the wet-mill stream can be steep liquor, light steep water, heavy steep liquor or mixtures thereof.

In some embodiments the process includes recycling the aqueous stream containing water-soluble carbohydrates. In other words, contacting the aqueous stream containing water-soluble carbohydrates with the protein-containing material and the carbohydrases and then separating the aqueous stream containing water soluble carbohydrates from the protein concentrate.

In some embodiments protein-containing material used in the processes described herein can be the light gluten fraction, heavy gluten fraction, corn gluten concentrate, corn gluten meal, gluten cake and mixture thereof.

In yet other embodiments the carbohydrases can be reacted with the protein-containing material and the wet-mill stream at temperatures that are at least room temperature, at least 40°C, at least 50°C, at least 70°C, at least 90°C, at least 100°C, or at least 120°C.

The resulting protein concentrate and/or the aqueous stream containing water-soluble carbohydrates can be dried. The aqueous stream containing water-soluble carbohydrates can be dried to greater than 60%, greater than 70%, or greater than 80% dry solids.

The current invention further relates to a process for increasing recovery of proteins in one or more protein containing materials of grain wet milling process and

characterized in that in said process the content of water-soluble carbohydrates is increased in at least one aqueous stream containing water-soluble carbohydrates.

Furthermore, it relates to a process comprising the following steps :

a. Taking a protein containing material obtainable after at least one separation step in the wet-milling process,

b. Contacting an aqueous stream of said wet-milling process with the protein containing material,

c. Adding an effective amount of carbohydrase for converting starchy material in said protein containing material into water-soluble carbohydrates,

d. Separating in two streams, preferably a protein concentrate and an aqueous stream enriched with water soluble carbohydrates.

DETAILED DESCRIPTION

I. Methods of Making Products

The disclosed process can be used to make a protein concentrate from any grain that is wet milled, for example, corn, wheat, barley, or sorghum (millet).

a. Wet-milling

Wet-milling grain involves soaking the grain.

In the corn wet-milling process the soaking of the grain is termed "steeping." The steeping process of corn, generally, includes the addition of sulfur dioxide (from about 0.1 to about 0.3 %) and steeping times of from about 24 to about 48 hours at temperatures between from about 45 to about 60 °C. After steeping, light steep water is obtained, which contains a high percentage of the soluble parts from the corn kernels. The

resulting steeped corn kernels are relatively softer than they were prior to steeping and at the end of the steeping process they can be separated into germs, fiber, starch and proteins.

The steeped corn is course ground in two steps to release the germ from the kernels. The germs are separated after each coarse milling step. Germs have an oil content of approximately 45-55%. The oil is usually extracted in subsequent refining steps.

The remaining coarse de-germed kernels are milled for the third time to disrupt the endosperm matrix and release the starch. Fibers are removed from the starch and endosperm proteins by passing the slurry over a series of screens.

The separated fiber is then dewatered and dried. In some instances the fiber is combined with steep water that has been concentrated in evaporators until it reaches about 45 to about 50% dry solids. The dried mixture of fiber and steepwater is referred to as corn gluten feed.

The remaining starch protein mixture is thickened and separated using a series of centrifuges. In the mill stream thickener (MST) centrifuge, the feed density is increased to improve separation of starch and endosperm protein (gluten). The overflow from the MST is sent to the steep house for use as steep water. The underflow from the MST is sent to the primary centrifuge (primary separation step). In the primary separation step the gluten proteins are partially separated from the starch. The overflow from the primary centrifugation step is the light gluten stream. The primary underflow is sent to starch washing to purify the starch. Overflow from the starch wash step is thickened in the clarifying centrifuge. The clarifier underflow is returned to the primary centrifuge

feed tank. The clarifier overflow is used for primary centrifuge wash water and fiber wash water.

The light gluten stream, containing about 5 % dry solids, is concentrated in the gluten thickener centrifuge. The overflow is used for fiber and germ washing. The underflow, referred to as heavy gluten contains from about 10 to about 20 % of dry substance, mainly insoluble proteins (about 64 % on dry base) and from about 10 to about 25 % of starch (on dry base). The suspended solids in the heavy gluten are separated from the process water with rotary vacuum filters. The gluten cake that discharges from the filters contains about 55 to about 65% water. The process water (sometimes referred to as gluten filtrate) that was separated from the gluten cake is returned to the gluten thickener feed tank. The gluten cake is dried to a moisture content of about 10 to about 12%, and is referred to as corn gluten meal.

b. Starting Materials

The process of producing a corn protein concentrate can start using any corn-protein-containing material that is produced during the wet-milling process. The term "corn gluten" as used herein refers to water insoluble proteins derived from endosperm. The term "corn protein containing material" refers to streams generated from the wet-milling process wherein greater than 2% of the solids are gluten, and less than one quarter of the original kernel fiber and germ. For example, corn-protein-containing material includes streams such as heavy gluten, gluten cake, starch wash overflow, and primary feed. One or more of these corn-protein-containing materials can be used in the process.

The wet-mill stream is a flowable stream that has previously been used in the wet-milling process. Exemplary wet-mill streams include corn steep liquor (CSL), which can

be either heavy (evaporated CSL) or light (LSW), mill-stream thickener overflow or underflow, primary centrifuge overflow or underflow, or gluten overflow and gluten filtrate. These streams are characterized in that they have at least trace amounts of protein and carbohydrates from corn.

The carbohydrases used can be any enzyme that can facilitate the degradation (either saccharification or liquefaction) of a complex carbohydrate to a water-soluble carbohydrate. For example, enzymes such as alpha-amylase, glucoamylase, hemicellulases, and cellulases or mixtures can be used.

In some embodiments the protein content of the protein concentrate can be altered by using additional enzymes. For example, phytases and or pectinases can be used to digest the pectin and/or the phytate, which will allow them to be separated from the protein concentrate. Use of phytases and pectinases may also result in a protein concentrate that is more digestable than a concentrate that has not been treated. Other enzymes that may be used are enzymes that join protein fragments, for example polyphenoloxidases and/or transglutaminases. In some applications elongated proteins will be more desirable. These enzymes can be introduced simultaneously with the carbohydrases or they can be added in a separate step.

b. Processing

One or more of the corn protein containing materials is contacted with one or more wet-mill streams and one or more carbohydrases. The corn protein containing material, wet mill-stream and carbohydrases can be placed in contact with each other using any method known in the art, such as by slurring, mixing, or blending.

The composition containing the carbohydrases eventually supplemented with additional enzymes, wet-mill stream, and corn-protein-containing material is incubated at a time and temperature sufficient to at least degrade the starch and/or other complex carbohydrates present in the corn-protein-containing material and/or the wet-mill stream to the point where upon separation of the aqueous stream containing water soluble carbohydrates from the resulting corn protein concentrate, the aqueous stream has a higher concentration of water soluble carbohydrates then the wet-mill stream had prior to contacting the carbohydrases.

Exemplary temperatures that can be used to incubate the mixture containing the carbohydrases, wet-mill stream, and corn-protein-containing material include form about 30 to about 250°F (15-120°C), and exemplary incubation times include from about 1/2 hours to about 40 hrs. The incubation temperature and time depend on the starting materials, enzymes, and the amount of enzymes used.

Separating the protein concentrate from the aqueous stream can be accomplished by any method known in the art. For example, filtration, centrifugation, coagulation, and combinations thereof can be used.

It is also possible to increase the concentration of water-soluble carbohydrates in the aqueous stream by recycling, or reusing, the aqueous stream as one of the wet-mill streams used in the process.

The protein concentration of the resulting protein concentrate can additionally be increased by rinsing the resulting concentrate with water, and/or a wet-mill stream. The rinsing washes away residual carbohydrates and increases the protein concentration on a

dry basis. Using this technique the protein concentration can be increased by at least 2, 5, 7, or 10% on a dry basis.

Yet another way of increasing the concentration of protein in the protein concentrate is to remove fats from the concentrate (defatting). Defatting can be accomplished using any method known in the art, for instance by using one or more solvents and/or degrading the fats with enzymes. Examples of solvents that can be used include hexane, isohexane, alcohols and mixtures thereof. Examples of enzymes that can be used include lipases and the like. The fats can subsequently be separated from the protein concentrate using any method known in the art, for example filtration, floatation, and/or centrifugation.

Additionally, the protein concentrate can be decolorized by bleaching using either chemical and/or enzymatic methods. Enzymes that can be used to facilitate bleaching include those having lipoxygenase (LOX) activity, and peroxidase activity. Chemicals that can be used alone or in combination with enzymes to facilitate bleaching include ozone, persulfate and peroxides.

II. Uses of the Products

The resulting aqueous stream containing water-soluble carbohydrates and/or the protein concentrate can be used as the sole carbon and nitrogen source for various fermentations or it can be blended with other carbon sources to provide a cost efficient fermentation.

In some embodiments, the aqueous stream containing water-soluble carbohydrates can be concentrated to dry substance levels of about 60 % and higher.

The products produced can also be used in feed applications for hogs in a liquid form or for cattle and poultry in a dried form.

The protein concentrate can be used in various animal feeds (including but not limited to farm animals, companion animals, fish, humans, and exotic animals) for improving the digestibility. The concentration of protein in the protein concentrate allows for the delivery of a desired amount of protein without having to deliver a large volume of material. This lessens the amount of waste produced by the animal and can contribute to digestibility improvement.

The protein concentrate can also be used as a food texturizer and flavor modulator in products that will be consumed by humans.

Additional features and advantages of the invention will be described in and apparent from the examples provided below.

EXAMPLES

Example 1.

Analytical Procedures :

Dry solids were determined by drying a test portion of the material at 103 °C during 4 hours using a method adapted from Dutch standard method NEN 3332.

Total and soluble protein content were determined according AACC method 46-30 using the Dumas method by combustion of a sample at a minimal temperature of 950 °C in pure oxygen and determination of nitrogen using a thermal conductivity detector. For nitrogen to soluble or total protein conversion factor of 6.25 was used.

Starch content was determined by a method derived from AACC 76-13 and the Megazyme kit method for starch. Samples were washed with ethanol to remove sugars. Solubilization of starch is achieved by cooking the sample in the presence of thermo stable α-amylase followed by amyloglucosidase. The glucose formed is measured using glucose oxidase/ peroxidase reagent and measurement of the absorbance.

Total starch carbohydrates are determined by the previous method but washing of sugars with ethanol is skipped. The difference between total starch carbohydrates and starch content results in the amount of soluble sugars.

Sugars were determined on a DP-4 column using a method derived from AACC 80-05 and determined as the sum of the quantitation of glucose, fructose, maltose and maltotriose standardized against the column.

Total or crude lipid content is determined using a method derived from AACC 30-24, 30-20, 30-25 and EEC method L25/29 using a Soxtec extraction instrument. A thimble containing a sample is immersed directly into boiling petroleum-ether. The thimble is moved above the solvent to the rinse-extraction step. Finally, after evaporation of the solvent, the residue is weighted and the lipid content is calculated.

Organic acid content is determined by HPLC method using UV detection.

Ash is determined using a method derived from AACC 08-01 by wet-ashing of a sample at 900 °C.

Phytate is determined in the sample by extraction of phytic acid and purification using different techniques and analyzed quantitatively by HPLC using conductivity.

EXAMPLE 1. Gluten cake with light steep water (lab procedure)

Experimental Procedures:

Heavy gluten slurry (HGS) and light steep water draw off (LSW) were collected from two different European Cerestar corn wet milling facilities (further referred to as CWM 1 and CWM 2). Dewatered corn gluten cake (CG cake) was obtained by filtrating heavy gluten slurry over a Buchner filter or were collected directly from these corn wet milling facilities. Heavy gluten slurry contains solubles that were removed by this filtration step. The proximate composition of the mill streams is listed in Table 1a-1.

Table 1-1. Proximate composition of mill streams used.

Mill Stream	Dry solids (%)	Protein (% db)	Starch (% db)	Sugars (% db)
HGS CWM 1	15.7	60.7	20.8	4.5
CG cake CWM 1	34.1	63.3	20.9	1.5
CG cake CWM 2	41.1	68.0	11.4	2.9
LSW CWM 1	10.5	43.7	1.7	6.8
LSW CWM 2	10.8	41.1	3.9	8.2

The dewatered corn gluten cake was mixed with light steep water (LSW) in ratio of 1:3, 1:4, 1:5, 1:6, 1:7, and 1:8 (CG cake: LSW). Generally, as the dry solid content increases the reaction times should be increased to get sufficient destarching.

For clarity, the following description of the process is provided as it was used for a sample having a ratio of 1:6 (CG cake:LSW). A mixture of 1800 mL of freshly taken light corn steep water (dry solids content 10.5 –10.8%) and 300 g of dewatered corn gluten cake (35-40 % of dry solids – see Table 1a-1 for appropriate number) was stirred for 30 minutes at 50 °C. At the end of this period the pH was adjusted with 10 % (m/m) sodium hydroxide to pH 5.8-6.2.

Liquefaction of the starch was achieved by adding 0.1 % of thermostable α-amylase (e.g. Termamyl ™, Novozymes A/S, DK-2880 Bagsvaerd, Denmark) on dry matter to the slurry and heating at 95 °C for 15 minutes. The viscosity increased and after cooling to 60 °C and adjusting the pH to 4.7 with 10 % (m/m) hydrochloric acid, 0.1 % of gluco-amylase (e.g. Glucostar 300L, Dyadic International Inc., Jupiter, Florida, USA) on dry base added and incubation was continued for at least two hours.

In one case, also 300 mg of phytase (Finase, AB Enzymes, Darmstadt, Germany) was added to the slurry and the temperature was then kept at 50°C for 2 hours. It was convenient to do this step at the same time as the addition of gluco-amylase.

Then the mixture was filtered on a Buchner funnel under vacuum using a Wattman 91 [Whatman PLC, Maidstone, Kent, UK] filter resulting in a gluten cake and a filtrate. The gluten cake was dried at 80 °C during 20 minutes. The filtrate was evaporated on a rotary vacuum drier at 40°C and could easily be concentrated to a dry solid content of about 70 %.

The results of the composition of the products are summarized in Table 1.2.

Table 1.2. Results of corn gluten concentrate and filtrate of two different corn wet mills (ratio 1:6) (All numbers on dry base)

Component	Corn gluten concentrate		Filtrate	
	CWM 1	CWM 2	CWM 1	CWM 2
Starch	0.5	2	0.5	0.5
Total protein	68	75	30	35
Lipids	5	4	n.d.	n.d.
Sugars	3	3	15	30
Phytate	1	1	3	3
Organic acids	9	4	30	18
Crude fiber	10	8	n.d.	n.d.
Ash	3.5	3	22	11

Results/Discussion:

Dewatering heavy gluten slurry results in a gluten cake containing with a higher protein content and less solubles. After liquefaction and saccharification the cake consisting of the corn gluten concentrate has a higher dry solid content (40- 45 % range) compared to the starting CG cake (35-40 %). Differences in the composition of LSW and CG cake also results in somewhat different composition of both the protein concentrates and filtrate. Compared to heavy steep water these filtrates can be easily evaporated to a higher dry solid content to 60-70 %. This is much higher then with heavy corn steep liquor that usually can be concentrated in the range of 45 to 50 % of dry solids. Composition of these filtrates is different compared to steep water : less proteins (30 –35 %) and more sugars (15 to 30 %) depending on the composition of the starting LSW (40 % proteins and up to 15 % sugars).

EXAMPLE 2. Enzymatic bleaching of heavy gluten slurry (lab procedure)

Experimental Procedures:

In these experiments untoasted full fat soy flour (Provaflor) from Cargill, Ghent (Belgium) containing active lipoxygenase (LOX) at different substitution levels to heavy gluten slurry (HGS) was used.

Bleaching of xanthophylls in HGS was carried out at room temperature. A portion of 75 g heavy gluten slurry was weighed into a 200 ml beaker. Water was added (10 ml) to dilute the slurry and the pH was adjusted to 6.5 by adding dropwise 1.0 M NaOH. Then soy flour was added at 5 and 15 % substitution levels for heavy gluten (w/w, db) and the mixture was incubated for 1 hour at 40 °C. During incubation the slurry was stirred at 2000 rpm using a magnetic stirrer with a stirrer bar and air was passed

continuously through the slurry using an aquarium and pond air pump. Upon completion of the reaction, the pH of the slurry was immediately readjusted below 2.5 by adding 1.0 M HCl to stop enzyme activity. Finally, the samples were lyophilized and analyzed on xanthophylls using a spectrophotometric method.

Table 2-1. Results of xanthophylls level using full fat soy flour.

Product	HGS freeze dried	5 % soy flour	15 % soy flour
Xanthophylls content (ppm)	162	63	40
Colour level compared to freeze dried HGS	100 %	38 %	25 %

Results/Discussion:

At a substitution level of 5% soy flour (w/w, db) 62 % of xanthophylls in the heavy gluten slurry were bleached whereas 75% were bleached at a substitution level of 15% soy flour (calculated against the freeze dried starting material).

For those skilled in the art it is clear that the same procedure can be used starting from the dewatered gluten cake and that LSW or demi-water needs to be added then to have a slurry that can be oxygenated in an appropriate way.

EXAMPLE 3. Enzymatic bleaching of heavy gluten slurry (pilot plant procedure)

Experimental Procedures:

Heavy gluten slurry (HGS) was collected from two different Cerestar corn wet milling facilities (further referred to as CWM1 and CWM2). The same procedure can be applied on CG cake but for convenience of trials heavy gluten slurry was used. The proximate composition of the heavy gluten slurry is listed in Table 3-1.

Table 3-1. Proximate composition of heavy gluten slurry used.

Plant	Dry solids (%)	Protein (% db)	Starch (% db)	Crude lipids (% db)	Xanthophylls (ppm)
CWM 1	15.7	60.7	20.8	6.7	215
CWM 2	12.2	60.4	12.5	8.4	217

Heavy gluten slurry was adjusted to pH between 5.8 to 6.2 and 0.1 % of thermostable α-amylase (e.g. Termamyl 120L from Novozymes A/S, DK-2880 Bagsvaerd, Denmark)) was added on dry matter base at ambient temperature. This mixture was pumped at a flow rate of 80 L/h through the liquefaction unit with a steam injector operating at steam pressure of 7.5 bar. Product pressure was 10 bar at 100 °C and mixture had a holding time of 15 minutes (back pressure of 1 bar).

Bleaching is preferentially conducted before liquefaction, but after reaction with thermostable α-amylase. After cooling to ambient temperature the pH of the mixture was adjusted to about 6.5 to 7. Freshly produced full fat soy flour (Provaflor, Cargill Ghent) was added to the heavy gluten in a 5 % amount based on the dry matter of the heavy gluten. Bleaching was conducted in a Belginox reactor with water heater and circulation in 300 kg batches. Conditions were as follows : stirring at 50 rpm, temperature 40 °C and air bubbling at 3 bar pressure rate during at least one hour. It was found out that increasing the time of bubbling had an improved bleaching result.

The liquefact was collected in a 600-litre tank and cooled to about 60°C. The pH was adjusted between 4.6 and 4.8 and 0.1 % of gluco-amylase (e.g. Glucostar 300L from Dyadic International Inc., Jupiter, Florida, USA) on dry matter base was added. Incubation time varied depending on the scale from 2 to 15 hours.

In order to filtrate the hydrolysed starch sugars, the reacted slurry needed to be diluted with about the same amount of demi water at 60 °C before pumping to the filtration unit at a rate between 250 to 300 L/h. Filtration was continued till the pressure was 12 bar. The last step in the filtration operation was the supply of pressured air of 5 to 6 bar to the filter press to dry the cake till no more filtrate left the press.

The gluten cake of about 40 % dry solids was dried on either a fluid bed dryer or a ring dryer till about over 90 % of dry solids. With the fluid bed dryer operated at 70 °C of drying air some coarser product was obtained. The ring dryer needed to start up with about 5 kg of fluid bed dried material. Rate of feeding was adjusted manually to obtain a powder of about 90 % of dry solids.

Ten different batches of corn gluten concentrates were made and the analytical composition is summarized in Table 3-2.

Table 3-2. Results of analytical composition of different batches of bleached corn gluten concentrate.

Batch	Dry solids (%)	Protein (% db)	Starch (% db)	Crude lipids (% db)	Xanthophylls (ppm)
CWM 1-1	95.3	75.3	0.1	10.1	154
CWM 1-2	95.1	77.5	0.2	9.9	155
CWM 1-3	96.7	77.6	< 0.5	10.0	130
CWM 1-4	94.3	75.3	0.8	10.0	152
CWM 1-5	93.6	75.0	0.8	9.5	147
Average 1-5	**95.0**	**76.1**	**0.4**	**9.9**	**148**
CWM 2-6	93.6	80.0	0.2	10.1	163
CWM 2-7	91.1	76.7	0.8	10.6	159
CWM 2-8	93.6	81.5	0.4	10.1	206
CWM 2-9	95.4	79.2	0.1	10.3	127
CWM 2-10	94.3	77.1	0.8	10.6	118
Average 6-10	93.6	78.9	0.5	10.3	155

Results/Discussion:

While on lab scale degree of bleaching between 60 to 70 % was obtained, the pilot plant trials resulted in products with about 30-35 % of bleaching. Corn gluten concentrates bleaching according the pilot plant method resulted in xanthophylls levels of about 150 ppm.

Resulting products had protein levels above 75 % on dry base and starch content below 0.5 %. After destarching the lipid levels of the resulting corn gluten concentrates are clearly higher compared to the starting heavy gluten slurries.

EXAMPLE 4. Coagulation procedure (lab procedure)

Experimental Procedures

Dewatered corn gluten cake (CG cake) and light steep water draw off (LSW) were collected from a European Cerestar corn wet milling facility. Samples of these streams were mixed in a ratio of 1:3.85 (CG cake:LSW) (about 15 (m/m)% db) at a temperature of 50°C with a residence time of 30 minutes. Dry solids of LSW and CG cake were measured on with an IR balance.

Liquefaction was started after adjusted the pH to 5.8-6.2 using 10 (m/m)% NaOH. Termamyl ™ (Novozymes A/S, DK-2880 Bagsvaerd, Denmark) was added to the mixture (0.1 (m/m)% on dry basis), the temperature was incrementally increased to 100°C. Once the temperature was above 93°C, the 15 minutes residence time took off.

For saccharification the mixture was cooled to 60°C and the pH was adjusted to 4.7 and 0.1 (m/m)% dry base of gluco-amylase Glucostar 300L (Dyadic International Inc., Jupiter, Florida, USA) was added and the resulting mixture was incubated for 120 minutes.

Coagulation was accomplished by adjusting the pH of aliquots of the liquefied and saccharified slurry by using either 4M HCl or using a 10 (m/m)% sodium hydroxide solution to adjust the pH ranging from 4.0 to 6.0 in steps of 0.5 while stirring. Separation of the unsoluble fraction from the solubles was achieved by filtration on a Buchner funnel under vacuum using a Wattman 91 [Whatman PLC, Maidstone, Kent, UK] filter. Yields of filtrate and cake were determined and dry solids content was determined with an IR balance. A gluten cake with about 40 % of dry solids was obtained. The protein content and amount of precipitate were determined. The filtrate was evaporated under vacuum (at least 30 mbar) on 40°C in rotary evaporator. The gluten cake with the wet corn gluten concentrate (~ 40 % dry solids) is cut over a 1 mm sieve and dried in 20 minutes in a laboratory fluid bed drier on 80°C maximum.

The resulting products were analyzed and the results of the coagulation experiments were summarized in Table 1d-1.

Table 4-1. Results of coagulation experiments

pH Coagulation	Dry base yield (%) (Col 1)	Soluble Protein ((m/m)% db) (Col 2)	Insoluble Protein ((m/m)% db) (Col 3)	Total Protein (m/m)% db (Col 4)	(Col 1)* (Col 2) %
4.0	82.2	2.8	67.7	70.6	55.7
4.5	88.7	13.9	55.6	69.5	49.3
5.0	87.0	7.3	61.6	68.9	53.6
5.5	87.7	7.4	62.9	70.3	55.2
6.0	90.0	15.7	51.5	67.2	46.3

Results/Discussion:

It is clear that at pH between 5 to 5.5 yields are higher compared to 4.5 or 6.0. For those skilled in the art it is known that better results are obtained when temperature is increased for a certain period of time to induce thermo coagulation of proteins.

EXAMPLE 5

Experimental Procedures:

Dewatered corn gluten cake (CG cake), corn gluten dewatering filtrate (CG filtrate), and light steepwater draw off (LSW) were collected from the Cargill, Inc. wet milling facility, Dayton, OH, USA. The proximate composition of the three mill streams is listed in Table 5-1.

Table 5-1. Proximate composition of mill streams used.

Mill Stream	Dry solids (%)	Protein (% db)	Sugars (% db)
CG cake	38.9	70.9	9.0
CG filtrate	2.3	65.8	15.1
LSW	8.2	43.8	23.0

Three different mixtures of CG cake were made with distilled water, CG filtrate, or LSW. Three different mixtures of cake and liquid were made by combining 693 grams cake with either: 1) 1340 grams of distilled water, 2) 1449 grams of LSW, or 3) 1372 grams of CG filtrate. To each of the three mixtures an amount of 16% (w/w) sodium hydroxide was added to adjust the pH of the mixture to 5.60. To each mixture, alpha-amylase (Fred L from Genencor, Beloit, WI, USA) was then added at an amount of 0.065% (w/w) per dry solids of the mixture and mixed into the mixtures. Each mixture was then placed in a separate 4-liter plastic jar and incubated in a 90°C water bath for 3 hours. Each jar was mixed approximately every 15 minutes by shaking. After the 3 hours of incubation were complete, each of the 3 jars was cooled to 60°C in a cold water bath and a sufficient amount of 11% sulfuric acid was added to each mixture to adjust the pH to 4.3. To each mixture, Optimax 4060VHP glucoamylase (Genencor, Beloit, WI, USA) was added at an amount of 0.065% (w/w) per dry solids content to each mixture. Each

mixture was then placed in a shaking incubator at 60°C for a period of 40 hours. After the 40 hours of incubation, each jar of each mixture was cooled to 30°C in a cold water bath. Therein, each mixture was vacuum filtered using a Whatman #3 paper (Whatman, Clifton, NJ, USA) sufficient to produce a cake of approximately 35% dry solids and the filtrate was collected. An amount of water approximately equal to the cake mass was added on the surface of the cake as it became visually dewatered.

Each of the obtained cakes were placed in a 103°C air oven and dried according to the official analytical method AACC 44-15A of the American Association of Cereal Chemists. The particle size of the cake was reduced by grinding in a coffee grinder and protein content was determined by the official analytical method AACC 46-30 of the American Association of Cereal Chemists. A total nitrogen to crude protein conversion factor of 6.25 was used.

The sugar content of the mill streams and the collected filtrate from the three mixtures was determined by filtering each liquid fraction through a 0.45 micron Whatman syringe filter and injecting the liquid into an HPLC system consisting of an Aminex HPX-87H ion exclusion column (Bio-Rad, Hercules, CA) with a 0.01N sulfuric acid mobile phase eluded at 0.6 ml/min and a Waters model 410 Refractive index detector (Waters Corporation, Milford, MA, USA). Analysis of the obtained information was made using Waters Millenium software. The sugar content was determined as the sum of the quanitation of glucose, fructose, maltose and maltotriose sugars standardized against the column.

Table 5-2. Results of Products Obtained

Product	Mixture	% Protein (db)	% Sugars (db)
Cake	CG cake + distilled water	85.45	--
Filtrate	CG cake + distilled water	15.8	76.8
Cake	CG cake + CG filtrate	86.7	--
Filtrate	CG cake + CG filtrate	23.5	63.5
Cake	CG cake + LSW	84.7	--
Filtrate	CG cake + LSW	40.3	33.3

Results/Discussion:

From comparing the composition of the initial millstreams presented in Table 5-1 and the composition of the final products obtained from each of the initial three mixtures presented in Table 5-2, it is apparent that the protein content of the corn gluten cake was increased from about 70.9%(db) to about 85.5%(db) for the water mixture, to about 86.7%(db) for the CG filtrate mixture, and to about 84.7%(db) for the LSW mixture. Additionally, the sugar content of the CG filtrate liquid stream was increased from about 15.1%(db) to about 63.5%(db), and the sugar content of the LSW filtrate stream was increased from about 23.0%(db) to about 33.3%(db). From this data it is apparent that this invention can be used to increase the protein content of CG cake while also increasing the sugar content of another water or mill stream mixed with and then later separated from the CG cake.

EXAMPLE 6

Experimental Procedures:

Clarifier centrifuge underflow (ClrUF), gluten thickener centrifuge overflow (GTOF), corn gluten dewatering filtrate (CG filtrate), and light steepwater draw off (LSW) was collected from the Cargill, Inc. wet milling facility, Dayton, OH, USA. The ClrUF was dewatered and formed into a cake of 29.8% solids by vacuum filtering over a Whatman #3 filter paper. The proximate composition of the ClrUF cake and the other three mill streams is listed in Table 6-1.

Table 6-1. Proximate composition of mill streams used.

Mill Stream	Dry solids (%)	Protein (% db)	Sugars (% db)
ClrUF cake	29.8	6.6	10.5
GTOF	2.4	60.1	25.4
CG filtrate	2.3	65.8	15.1
LSW	8.2	43.8	23.0

A mixture of ClrUF cake and GTOF was made by mixing an amount of 1131 grams cake with 1500 grams of GTOF. The ClrUF cake and GTOF mixture was adjust to pH 5.60 by adding 7.73g of 16% (w/w) sodium hydroxide. To the mixture, an amount of 360 microliters of Fred L alpha-amylase (Genencor, Beloit, WI, USA) was then added and mixed into the mixture. The mixture was then placed in a 4 liter plastic jar and incubated in a 90°C waterbath for 2 hours. A mixing impeller was submerged into the mixture within the jar, connected to a variable speed drive, and rotated at approximately 200 rpm. After the 2 hours of incubation were complete, the jar was cooled to 30°C in a cold water bath. Therein, the mixture was vacuum filtered over a Whatman #3 paper,

Whatman, Clifton, NJ, USA, sufficient to produce a cake of approximately 30% dry solids and the filtrate was collected. Four different cakes where produced. The first cake was vacuumed filtered only and filtrate was collected without wash water addition. The second cake was similarly filtered and then washed with an amount of water approximately equal mass to the cake mass was added on the surface of the cake as it became visually void of liquid water. The third, fourth, and fifth millstreams were similarly filtered and washed with GTOF, CG filtrate, and LSW, respectively.

Each of the obtained cakes were placed in a 103°C air oven and dried according to the official analytical method AACC 44-15A of the American Association of Cereal Chemists. The particle size of the cake was reduced by grinding in a in a coffee grinder and protein content was determined by the official analytical method AACC 46-30 of the American Association of Cereal Chemists. A total nitrogen to crude protein conversion factor of 6.25 was used.

The sugar content of the mill streams and the collected filtrate was determined by filtering each liquid fraction through a 0.45 micron Whatman syringe filter and injecting the liquid into an HPLC system consisting of an Aminex HPX-87H ion exclusion column (Bio-Rad, Hercules, CA) with a 0.01N sulfuric acid mobile phase eluded at 0.6 ml/min and a Waters model 410 Refractive index detector (Waters Corporation, Milford, MA, USA). Analysis of the obtained information was made using Waters Millenium software. The sugar content was determined as the sum of the quantitation of glucose, fructose, maltose and maltotriose sugars standardized against the column.

Table 6-2. Results of Products Obtained

Product	Millstream Used for Wash	% Protein (db)	% Sugars (db)
Cake	None added	41.4	--
Filtrate	None added	5.2	68.3
Cake	distilled water (control)	63.9	--
Cake	GTOF	60.6	--
Filtrate	GTOF	5.7	25.8
Cake	CG filtrate	59.6	--
Filtrate	CG filtrate	3.7	25.3
Cake	LSW	60.4	--
Filtrate	LSW	6.9	33.6

Results/Discussion:

From comparing the composition of the initial millstreams presented in Table 6-1 and the composition of the final products obtained as presented in Table 6-2, it is apparent that the protein content of the ClrUF cake was increased during practice of the invention from about 6.6%(db) to about 41.4%(db) with practice of the invention and when a washing step was not performed, and to about 63.9%(db) when wash water at an about an equal mass as the mass of the cake was added to the cake during filtering. When millstreams were used to wash the cake, the protein content of the cake was increased to about 60.6%(db) with the GTOF wash, to about 59.6%(db) with the CG filtrate wash, and to about 60.4%(db) with the LSW wash. Additionally, the sugar content of the GTOF which was captured as liquid stream/filtrate stream from the invention was increased

from about 25.4%(db) to about 68.3%(db) without any wash. The protein content of the filtrate also varied with the use of and between different mill streams used for washing. From this data it is apparent that this invention can be used to increase the protein content of the ClrUF mill stream while also increasing the sugar content of another mill stream mixed with and then later separated from the ClrUF cake, with or without the addition of another millstream or same used for washing the concentrated protein cake. The final sugar content of the filtrate from the process can be raised or lowered by the sugar content of the wash stream. Use of water for washing did not introduce other solubles into the filtrate thus preserving the sugar content of the stream. However, when GTOF or CG Filtrate with a lower sugar content (%db) were used, the final sugar content of the filtrate was reduced.

The wash stream used for washing the cake influenced the final protein content of the cake. It is obvious from Table 6-2 that a washing step increases the final protein of the cake as no washing step produced a cake with only 41.4% protein, but use of any of the other streams produced a cake of approximately 60% protein or greater. Water, which has no non-protein solubles, produced the highest protein cake of 63.9% (db). Other streams which contain solubles other than protein produced lower protein cakes (59.6-60.6% db protein). It is also obvious from these results that the final protein content of the produced cake can be controlled by the protein content and non-protein dry matter content of the liquid used to wash the cake.

Example 7

Experimental Procedures:

The filtrate obtained from example 3 after filtering and without washing was further saccharified. To the filtrate a sufficient amount of 11% sulfuric acid was added to adjust the pH to 4.3. An amount 0.065% (w/w) per dry solids content of the filtrate of Optimax 4060VHP glucoamylase, Genencor, Beloit, WI, USA, was added to the filtrate. The filtrate was then placed in a shaking incubator at 60°C for a period of 40 hours. The sugar content of the mill streams and the collected filtrate was determined by filtering each liquid fraction through a 0.45micron syringe filter, Whatman, Whatman, Clifton, NJ, USA, and injecting the liquid into an HPLC system consisting of an Aminex HPX-87H ion exclusion column (Bio-Rad, Hercules, CA) with a 0.01N sulfuric acid mobile phase eluded at 0.6 ml/min and a Waters model 410 Refractive index detector, Waters Corporation, Milford, MA, USA. Analysis of the obtained information was made using Waters Millenium software. The sugar content was determined as the sum of the quantitation of glucose, fructose, maltose and maltotriose sugars standardized against the column.

Table 7-1. Sugar Content of Filtrate before and after Saccharification

Mill Stream	Sugar (%db)
Initial	68.3
Saccharified	91.5

Results/Discussion:

Saccharification with glucoamylase increased the concentration of sugar in the filtrate from about 68.3% (db) to about 91.5% (db), measured as the sum of glucose, fructose, maltose, and maltotriose. Thus indicating that larger chained carbohydrates can be isolated from a mill stream containing gluten by only liquifying and filtering away the gluten protein. These carbohydrates were then more fully hydrolyzed to smaller sugars measured by the HPLC using a saccharification step after separation from the gluten protein.

Example 8

Experimental Procedures:

One milliliter of a 24 h S. cerevisiae culture was inoculated into basal media containing 5 g/L peptone and 3 g/L yeast extract and 16 (Glucose A) or 50 g/L (Glucose B) of D-glucose (Sigma-Aldrich Co., St. Louis, MO) or prototype mill stream product added to generate starting glucose concentrations of 25 (Experimental A) or 45 g/L (Experimental B). The prototype product examined was the saccharified filtrate resulting from Example 7. Cultures were incubated at 30° C with 100 rpm shaking. Samples were taken after 0, 18 and 44 h and optical density at 595 nm was measured. Organic acid and ethanol profiles were quantitated by HPLC using an Aminex HPX-87H ion exclusion column (Bio-Rad, Hercules, CA) with a 0.01N sulfuric acid mobile phase.

Table 8-1. Optical Density and Ethanol yield of Fermented Glucose Control and Filtrate.

	O.D. 595 - T18	% Ethanol yield - T44
Glucose A	14.82	43.22
Glucose B	14.88	44.42
Experimental A	18.33	45.72
Experimental B	15.72	44.49

Table 8-1. Optical density measurements at 595 nm after 18 hours and ethanol yield after 44 h of *S. cerevisiae* growth. The Glucose A culture contained 17 g/L glucose and the Glucose B culture contained 50 g/L glucose in the basal peptone and yeast extract media. The Experimental cultures contained basal medium plus the prototype material normalized to 25 g/L (A) and 45 g/L glucose (B).

Results/Discussion:

Experiments were set up to examine fermentability of the prototype mill stream product. Fermentability in this study was defined as capability of the feedstock to support growth of S. cerevisiae and sustain product formation, in this case ethanol. The prototype material, as indicated by optical density measurements in Table 5-1, supported cell growth. In addition, dextrose was fully utilized in all cultures after 44 h, indicating fermentation occurred. Ethanol yields on the prototype material were between 44 and 46%, which is near 90% of the maximum theoretical yield (50%). Thus, the prototype material was capable of supporting S. cerevisiae growth and ethanol production. The prototype product, a filtrate resulting from liquified and saccharified clarifier underflow cake resuspended with gluten thickener overflow, was able to support *S. cerevisiae* growth and ethanol production at different starting glucose concentrations.

Example 9

Dewatered corn gluten cake (CG cake), same as used in Example 5, was collected from the Cargill, Inc. wet milling facility, Dayton, OH, USA. The cake was mixed with the saccharified filtrate without washwater addition (sacc'd filtrate) produced in Example 7. The proximate composition of the CG cake and sacc'd filtrate listed in Table 9-1.

Table 6-1. Proximate composition of mill streams used.

Mill Stream	Dry solids (%)	Protein (% db)	Sugars (% db)
CG cake	38.9	70.9	9.0
Sacc'd filtrate	22.9	4.8	91.5

An amount of 100 grams CG cake was mixed with 250 grams of sacc'd filtrate produced in Example 4. To the three an amount of 16% (w/w) sodium hydroxide was added to adjust the pH of the mixture to 5.60. An amount of 40 micoliters of alpha-amylase (Fred L from Genencor, Beloit, WI, USA) was then added and mixed into the mixture. The mixture was then placed in a 0.5 liter plastic jar and incubated in a 90°C waterbath for 3 hours. A mixing impeller was submerged into the mixture within the jar, connected to a variable speed drive, and rotated at approximately 200 rpm. After the 2 hours of incubation were complete, the mixture was cooled to 60°C in a cold water bath and a sufficient amount of 11% sulfuric acid was added to each mixture to adjust the pH to 4.30. An amount of 40 microliters of Optimax 4060VHP glucoamylase (Genencor, Beloit, WI, USA) was added to the mixture. The mixture was then placed in a shaking incubator at 60°C for a period of 40 hours. After the 40 hours of incubation, the mixture

was cooled to 30°C in a cold water bath. Therein, the mixture was vacuum filtered using a Whatman #3 paper (Whatman, Clifton, NJ, USA) sufficient to produce a cake of approximately 32.5% dry solids and the filtrate was collected. An amount of water approximately equal to the cake mass was added on the surface of the cake as it became visually dewatered.

The obtained cake was placed in a 103°C air oven and dried according to the official analytical method AACC 44-15A of the American Association of Cereal Chemists. The particle size of the cake was reduced by grinding in a in a coffee grinder and protein content was determined by the official analytical method AACC 46-30 of the American Association of Cereal Chemists. A total nitrogen to crude protein conversion factor of 6.25 was used.

The sugar content of the mill streams and the collected filtrate from the three mixtures was determined by filtering each liquid fraction through a 0.45 micron Whatman syringe filter and injecting the liquid into an HPLC system consisting of an Aminex HPX-87H ion exclusion column (Bio-Rad, Hercules, CA) with a 0.01N sulfuric acid mobile phase eluded at 0.6 ml/min and a Waters model 410 Refractive index detector (Waters Corporation, Milford, MA, USA). Analysis of the obtained information was made using Waters Millenium software. The sugar content was determined as the sum of the quanitation of glucose, fructose, maltose and maltotriose sugars standardized against the column.

Table 9-2. Results of Products Obtained

Product	% Protein (db)	% Sugars (db)
Cake	84.4	10.9
Filtrate	4.9	93.3

Results/Discussion:

From comparing the composition of the initial millstreams presented in Table 6-1 and the composition of the final products obtained from each of the initial three mixtures presented in Table 9-2, it is apparent that the protein content of the corn gluten cake was increased from about 70.9%(db) to about 84.4%(db). Additionally, the sugar content of the sacc'd filtrate liquid stream was increased from about 91.5%(db) to about 93.3%(db). The sugar content of the filtrate was similar before and after addition of the washing step using water (data not shown), indicating that a high level of pure sugar is retained in the cake and that its removal can be manipulated with the amount of washing performed. From this data it is apparent that this invention can be used to increase the protein content of CG cake while also increasing the sugar content of another water or mill stream mixed with and then later separated from the CG cake. As presented in this example, this mill stream may be a recycled filtrate stream obtained from filtration of the final CG cake product.

CLAIMS

1. A process comprising:

(a) contacting one or more protein containing materials with one or more wet-mill streams and one or more carbohydrases to produce at least one protein concentrate and at least one aqueous stream containing water-soluble carbohydrates; and

(b) separating the protein concentrate from the aqueous stream containing water-soluble carbohydrates.

2. A process according to claim 1, additionally comprising defatting the protein containing material.

3. A process according to claim 2, wherein defatting the protein-containing material comprises contacting the protein-containing material with a solvent.

4. A process according to claim 2, wherein defatting the protein-containing material comprises contacting the protein-containing material with an enzyme.

5. A process according to anyone of claims 1 to 4, wherein the grain is corn and the one or more protein containing materials comprises gluten.

6. A process according to anyone of claims 1 to 5, wherein said process is comprising a bleaching step.

7. A process according to anyone of claims 1 to 6, wherein at least one of the one or more wet-mill streams is steep liquor, light steep water, heavy steep liquor or mixtures thereof.

8. A process according to anyone of claims 1 to 7, wherein the aqueous stream containing water-soluble carbohydrates is recycled and used as one of the one or more wet-mill streams in step (a).

9. A process according to anyone of claims 1 to 8 wherein at least one of the one or more protein-containing materials is selected from the group consisting of light gluten fraction, heavy gluten fraction, corn gluten concentrate, corn gluten meal, gluten cake and mixture thereof.

10. A process according to anyone of claims 1 to 9 wherein step a) is taking place at a temperature of at least room temperature, preferably at least 50°C, more preferably at least 70°C, most preferably at least 120°C.

11. A process according to anyone of claims 1 to 10, wherein said process comprises a membrane filtration step before and/or after step b) of said process.

12. A process according to anyone of claims 1 to 11, further comprising the step of drying the protein concentrate.

13. A process according to anyone of claims 1 to 12, wherein a least one of the one or more carbohydrases is selected from the group consisting of alpha amylase, glucoamylase, hemicellulase, cellulase and mixtures thereof.

14. A process according to anyone of claims 1 to 13, further comprising contacting the one or more protein-containing materials, one or more wet-mill streams, and/or one or more carbohydrases with one or more enzymes to join protein fragments.

15. A process according to anyone of claims 1 to 14, wherein at least one of the one or more enzymes are chosen from polyphenoloxidases and transglutaminases.

16. A process according to anyone of claims 1 to 15, further comprising contacting the one or more protein-containing materials , one or more wet-mill streams, and/or one or more carbohydrases with one or more pectinases.

17. A process according to anyone of claims 1 to 16, further comprising contacting the one or more protein-containing materials with one or more phytases.

18. A process according to claim 1, further comprising contacting the one or more protein containing materials, one or more wet-mill streams, and one or more carbohydrases with one or more pectinases.

19. A process comprising contacting one or more protein containing materials with one or more wet-mill streams and one or more carbohydrases to produce at least one protein concentrate and at least one aqueous stream containing water-soluble carbohydrates, wherein greater than 2% of the solids in the protein-containing material are gluten.

20. A process for increasing recovery of proteins in one or more protein containing materials of grain wet milling process and characterized in that in said process the content of water-soluble carbohydrates is increased in at least one aqueous stream containing water-soluble carbohydrates.

21. A process according to claim 20 and said process is comprising the following steps :

e. Taking a protein containing material obtainable after at least one separation step in the wet-milling process,

f. Contacting an aqueous stream of said wet-milling process with the protein containing material,

g. Adding an effective amount of carbohydrase for converting starchy material in said protein containing material into water-soluble carbohydrates,

h. Separating in two streams, preferably a protein concentrate and an aqueous stream enriched with water soluble carbohydrates.

22. An animal feed comprising the protein concentrate produced according to the process of anyone of claims 1 to 21.

23. A fish feed comprising the protein concentrate produced according to the process of anyone of claims 1 to 21.

24. A fermentation feedstock comprising the aqueous stream containing water-soluble carbohydrates produced according to the process of anyone of claims 1 to 21.

25. Aqueous stream containing water-soluble carbohydrates obtainable according the process of anyone of claims 1 to 21.

26. Use of protein concentrate obtainable according to the process of anyone of claims 1 to 21 in food applications as food texturizer and/or flavor modulator.

27. Use of protein concentrate obtainable according to the process of anyone of claims 1 to 21 in animal feed for digestibility improvement.

28. Use of protein fraction obtainable according to the process of anyone of claims 1 to 21 in pet food as food texturizer and/or flavor modulator.

29. Use of protein concentrate obtainable according to the process of anyone of claims 1 to 21 in fish feed for digestibility improvement.

30. Use of aqueous stream containing water-soluble carbohydrates according claim 25 as fermentation feedstock.

ABSTRACT

Disclosed are a process for contacting a protein containing material with a wet-mill stream. Animal feed, fish feed and fermentation stock and their uses are disclosed as well.